Journal of Medical Virology 59:520–526 (1999)

Epidemiological Features of Rotavirus Infection in Caracas, Venezuela: Implications for Rotavirus Immunization Programs

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The epidemiological features of rotavirus infection may be quite relevant for evaluation of the performance of a rotavirus vaccine in different settings, as well as for monitoring its impact during vaccination under routine conditions. This article describes some important issues regarding rotavirus epidemiology in Venezuela, where major field trials of rotavirus vaccine have been carried out. Rotaviruses was significantly more frequently observed in inpatient (43%) than in outpatient (21%) consultations for diarrhea in infants and young children. There was a high prevalence of rotavirus illness, regardless of socioeconomic conditions, but the risk of dehydration was greater among the lower socioeconomic groups. Rotavirus disease occurs yearround, with a slight seasonal pattern. Eighty-five percent of rotavirus-positive diarrheal episodes, as well as 86% of cases of dehydration due to rotavirus, occurred during the first year of life. However, rotavirus illnesses occur less commonly during the first months of life (0–2 months), which may be a result of protection by transplacental antibodies. The pattern of acquisition of rotavirus antibody was consistent with this age distribution of disease and with optimal age for vaccination. Thus, regional epidemiological characteristics of rotavirus infection may affect optimal performance of rotavirus vaccine. ***J. Med. Virol. 59:520–526, 1999.***

KEY WORDS: epidemiology; rotavirus disease; Venezuela

INTRODUCTION

Diarrheal illnesses are an important public health problem, particularly in developing countries. The consequences of such illnesses have a major impact because they result in high rates of mortality and morbidity, as well as the deterioration of nutritional status [Guerrant et al., 1992]. Until the discovery of rotavirus as a cause of gastroenteritis in 1973 [Bishop et al., 1973], most infantile diarrhea was not associated with any etiological agent. However, rotaviruses have now been implicated as the etiological agents of up to one-half of the moderate or severe diarrheal episodes occurring in children younger than 2 years of age. This virus causes the death of approximately 870,000 infants and young children and approximately 18 millions of moderate to severe episodes of diarrhea per year in children younger than 5 years of age in the developing countries [Institute of Medicine, 1986]. They also account for a large proportion of inpatient (14–41%) and outpatient (1–34%) consultations in hospital settings worldwide [Bern and Glass, 1994]. In Venezuela, rotaviruses are the most frequent cause of diarrheal hospitalization among young children, accounting for 30–50% of such outcomes [Pérez-Schael et al., 1991]. It has been estimated that rotavirus is responsible for 101,400 episodes and 18,000 hospitalizations per year in Venezuelan children younger than 2 years of age [Pérez-Schael, 1996].

Extensive efforts have been made to develop a rotavirus vaccine effective in preventing severe rotavirus disease [Kapikian et al., 1996; Glass et al., 1994; Pérez-Schael et al., 1991]. Recent data from trials in the United States, Venezuela, and Finland indicate that the rhesus rotavirus-based quadrivalent vaccine, developed by Kapikian et al. [1996] at the National Institutes of Health (NIH), United States, is safe and efficacious against severe rotavirus illness [Rennels et al.,

Grant sponsor: CONICIT; Grant number: MPS-RV-140031; Grant sponsor: FUVESIN.

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Accepted 23 March 1999

1996; Santosham et al. 1997; Pérez-Schael et al., 1997; Joensuu et al., 1997]. This vaccine was recently licensed, 25 years after the discovery of rotavirus. The epidemiological features of rotavirus infection may be very important in assessing the performance of a rotavirus vaccine in different settings, as well as for monitoring its impact during vaccination under routine conditions. This article describes some important issues regarding rotavirus epidemiology in Venezuela such as frequency and distribution of infection and disease by age, sex, and socioeconomic status, as well as seasonality, and severity. In addition, immunological markers of susceptibility are presented.

MATERIALS AND METHODS

Study Area and Subjects

The study population resided in Caricuao and adjacent areas, a middle to low socioeconomic urban area located in the southwest of Caracas, Venezuela, composed of marginal and very crowded neighborhoods. Most prenatal, obstetric, and pediatric care is provided by the hospital Materno Infantil de Caricuao, which has 87 maternity beds, 90 pediatric beds, and 24 beds for emergency use for patients admitted within less than 48 hours. Between 4,000 and 5,000 deliveries are attended each year in this hospital.

The study population consisted of children younger than 36 months of age with acute diarrhea who were brought to the hospital Materno Infantil de Caricuao, between January 1988 and December 1993. In this group, 2,101 were seen from Monday through Friday (7 a.m–12 p.m.) and were thus evaluated for rotavirus infection. During 1992 and 1993, a subgroup of 920 inpatients or outpatients with diarrhea were further evaluated for the following epidemiological and clinical features, using standard questionnaire administered by pediatricians: age, sex, and socioeconomic status. The modified Graffar method [Méndez-Castellano and De Méndez, 1986] was used, which includes the occupation of the father, educational level of the mother, income level, and sanitary conditions rated on a scale of 1–5 scale: 1 = upper class; 2 = upper to middle class; 3 = middle to low class; 4 = low class; and 5 = marginal class. Clinical signs and symptoms were recorded daily until they disappeared. A diarrheal episode was defined as three or more semiliquid or liquid stools or as one stool containing mucus and blood within a 24-hour period. In addition, an episode was considered separate when a period of 48 hours without symptoms had elapsed from a previous episode. Dehydration was defined according to the criteria of the World Health Organization [WHO, 1990]. During 1990 and 1991, 234 healthy infants younger than 1 year of age were studied for rotavirus antibody prevalence. Fisher's exact test (two-tailed) was used for statistical analysis.

Rotavirus Antigen Detection and Serotyping

Stools samples were examined for rotavirus by a confirmatory enzyme-linked immunosorbent assay (ELISA) [Kapikian et al., 1979]. Rotavirus-positive specimens were serotyped by monoclonal antibody ELISA as previously described [Flores et al., 1988; White et al., 1991]. The monoclonal antibodies used for serotyping were generously obtained from Drs. Taniguchi and Urasawa (KU-4 for serotype 1, S2-2G10 for serotype 2, YO-1E2 for serotype 3, and ST-2G7 for serotype 4), Dr. Greenberg (2C9, 5E8 for serotype 1), and Dr. Green (954/159 for serotype 3).

IgA and Neutralization Assays

During 1990 and 1991, serum samples were obtained from 234 healthy infants younger than 1 year of age to carry out IgA ELISA using the RRV strain as virus antigen, according to Losonsky et al. [1988], and plaque-reduction neutralization assay (PRN) for Wa virus (G1 serotype) as described previously [Hoshino et al., 1984]. This strain was selected as antigen because during that period, the most prevalent rotavirus serotype was G1. A continuous cell line derived from fetal rhesus monkey kidney (MA104) was used for the neutralization test; a 60% reduction in the number of plaques was considered to represent the presence of neutralizing antibodies.

RESULTS

Description of Study Site

From January 1988 through December 1993, 300,747 patients sought care for any cause at the hospital Materno Infantil de Caricuao, for a yearly mean of 50,125 (range: 40,662–63,161) consults per year. Of those visits, 68% (204,508) were outpatient and 32% (96,239) were inpatient visits. Diarrheal illnesses accounted for approximately 11% (5,741/50,125) of the mean yearly consultations, ranging from 4,026 (9%) to 6,642 (15%) episodes per year. A total of 206 deaths were registered during the 6-year period, for a mean of 34 deaths per year. Nine (4%) of the 206 deaths were associated with diarrheal illness.

Rotavirus Distribution by Age, Sex and Socioeconomic Status

Age distribution, sex, and socioeconomic status of the subgroup composed of 920 children are presented in Table I. Between 1992 and 1993, rotavirus was detected in 318 (35%) of 920 infant and young children with diarrheal episodes. Most rotavirus disease (85%) occurred during the first year of life, with a relative low frequency during the first 3 months of age; only 16% (48/397) developed such illness. The frequency increased with age as 47% (223/474) of infants aged 3–11 months were rotavirus positive. The frequency of rotavirus infection decreased slightly (35%; 45/127) throughout the second year, and declined markedly in the groups aged 2 years or older (17%) (Fig. 1). Rotavirus infection occurred with similar frequency in males (37%; 197/534) than in females (31%; 121/386; $P = 0.102$).

Socioeconomic status did not appear to influence the occurrence of rotavirus illness. For example, a similar proportion (41%; 38/92) of patients belonging to the

TABLE I. Characteristics of Study Population: 1992–1993

Characteristics	No. of children (%)
No. of children	920
Sex	
M	534 (58)
F	386 (42)
Age groups (mo)	
0–5	541 (59)
6–11	240 (26)
12–23	127 (14)
24–35	12 (1)
Socioeconomic status[a]	
1–2 (upper and upper middle class)	6 (1)
3 (middle to lower class)	86 (19)
4–5 (lower class and marginal class)	363 (80)

[a]Socioeconomic study was realized in 455 children evaluated during 1993. None children belonged to status 1.

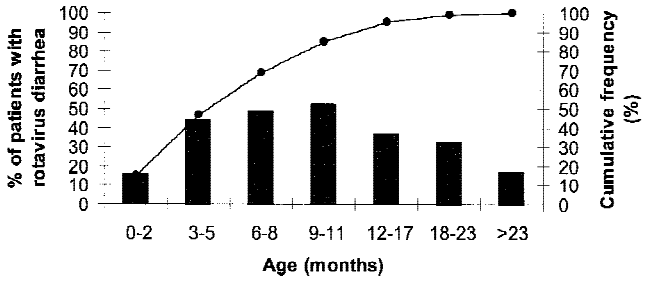

Fig. 1. Distribution and cumulative frequency of rotavirus episodes by age in Caracas, Venezuela.

middle class (status 2 and 3) and to the low and marginal class (40%; 146/363; $P = 0.905$), developed rotavirus illness. However, the proportion of dehydration due to rotavirus diarrhea was significantly higher in lower socioeconomic groups. In this study population, 44% (15/86), 47% (36/77), and 54% (37/69) of the children of status 3, 4, and 5, respectively, developed dehydration (44% vs. 47%, $P < 0.01$; 44% vs. 54%, $P < 0.001$; 47% vs. 54%, $P = 0.507$). There were significant differences between status 3 and 4 or 5 socioeconomic class groups, but not between status 4 and 5 groups. Very few patients belonged to high middle class group (status 2), none of whom developed an episode of dehydrating rotavirus (0/4).

Yearly Frequency and Seasonality of Rotavirus Illness

A total of 2,101 stool samples were tested for rotavirus over the 6-year period (1988–1993). The annual occurrence of rotavirus illness varied widely from 15% in 1989 to 39% in 1993, for a mean of 26% (549/2,101). The monthly pattern through 6 years is shown in Figure 2A. Whereas the cumulative percentage of rotavirus-positive episodes by months is shown in Figure 2B. Standard deviation and standard error varied each year from approximately 2–25% and 2–11%, respectively. The 95% confidence interval (CI) was stable for each month, except for September and December, which were negative and too wide. Although the number of episodes per year was variable and ranged from 186 in 1988 to 632 in 1993, rotavirus was present year-round, with a slight seasonal pattern observed throughout the 6-year period. Consistently, the percentages below the mean were observed during the rainy but warmest months, April through July (Fig. 2B). Analysis of quarterly distribution of rotavirus diarrhea during the 6-yearperiod showed similar frequency for each quarter, except for the April to June quarter (13%, 67/522). The proportions for other periods were as follows: January, February, and March (27%; 112/417); July, August, and September (31%; 227/705); and October, November, and December (31%; 143/457). The difference between the period April to June versus other periods were statistical significant ($P < 0.001$).

G serotype Distribution of Rotavirus Illness

Serotype G1 was the most prevalent G serotype (Fig. 3), accounting for 66% (337/509) of the strains that could be serotyped, followed by serotype G2 in 7% (38/509) and G4 serotype in 6% (31/509). Serotype G4 predominated during 1988 and 1989 (50% and 64%, respectively); meanwhile, for the next 4 consecutive years, 1990–1993, G1 was the most frequent serotype detected (81%, 80%, 43%, and 81%, respectively) and continued circulating during the vaccine study up to 1995 [Pérez-Schael et al., 1997]. Serotype G2 circulated concurrently with G1 in 1991 and 1992. Serotype G3 was not detected over the 6-year period. Twenty percent (103/509) of samples were untyped. Some of the untypable rotavirus strains (42/103) could belong to uncommon serotypes because they had sufficient number of double-shelled virus particles but did not react with any serotype or had insufficient double-shelled particles in the samples (61/103).

Clinical Signs and Symptoms of Rotavirus Disease

The signs and symptoms associated with diarrheal illness that were rotavirus positive and rotavirus negative were compared. As shown in Table II, rotavirus-positive diarrheal illnesses were associated with dehydration (47% vs. 29%; $P < 0.001$), vomiting (79% vs. 47%; $P < 0.001$), fever (57% vs. 33%; $P < 0.001$) and hospitalization (76% vs. 52%; $P < 0.001$) significantly more often than non-rotavirus diarrheal episodes. The presence of blood in the stool was significantly more often in patients with non-rotavirus diarrhea (22% vs. 8%; $P < 0.001$). Illness generally lasted less than 1 week in both groups, although a significantly greater number of children with rotavirus had episodes of less than 1-week duration than were experienced by children with non-rotavirus infection (89% vs. 83%; $P = 0.01$). Among the rotavirus-positive children (n = 318), the proportion of inpatients was significantly greater (76%) than the proportion of outpatients (24%; $P < 0.001$). This difference was not found among the non-rotavirus group (48% vs. 52%; $P = 0.05$). Noteworthy, rotavirus was significantly more frequently detected among in-

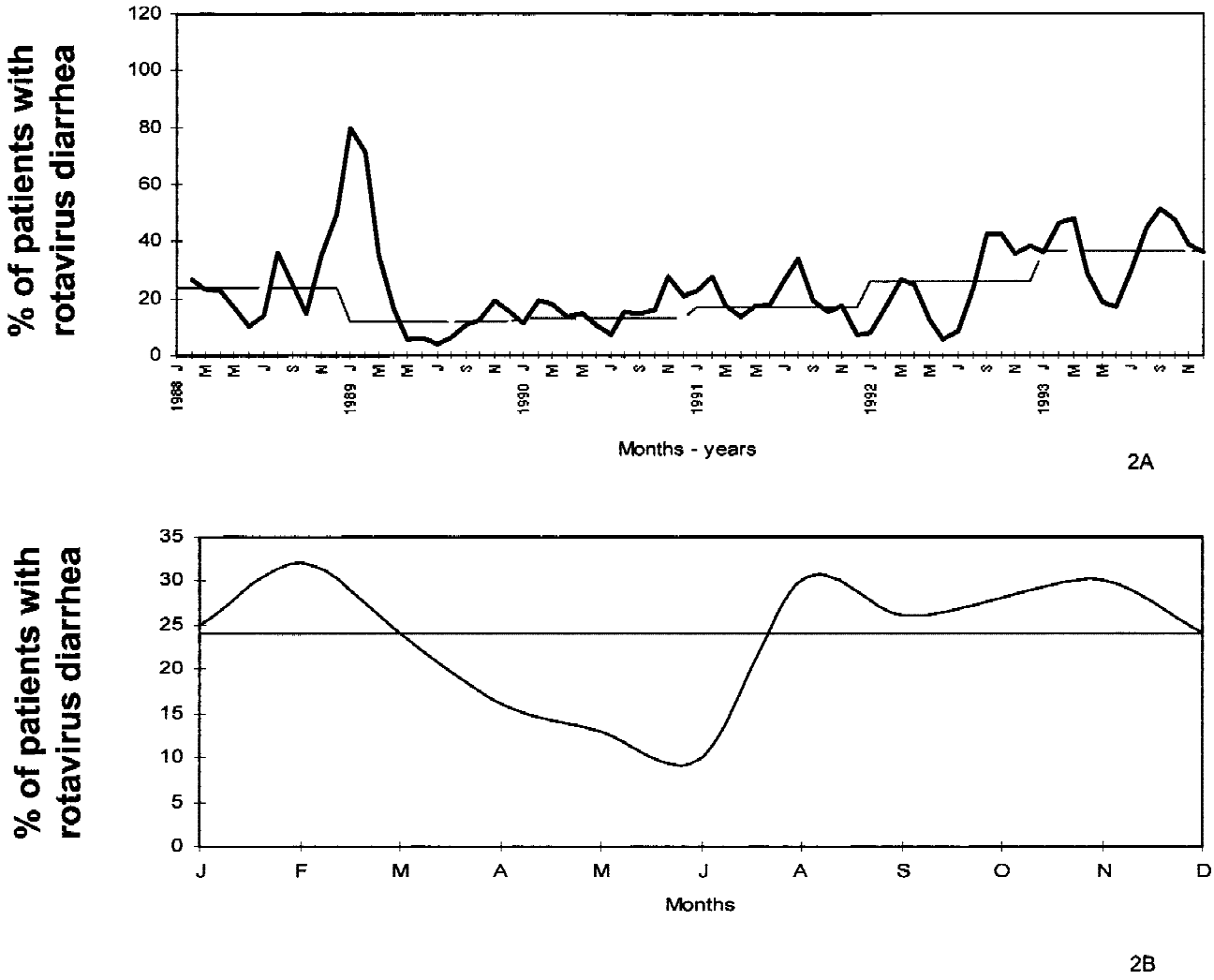

Fig. 2. Monthly detection for rotavirus illness in Venezuelan children younger than 3 years of age from 1988 to 1993. Horizontal lines in both represent annual means of percentages.

patients (43%; 241/557) than among outpatients (21%; 77/363; $P < 0.001$).

Dehydration due to rotavirus diarrhea decreased with age, with the lowest proportion of dehydration occurring in the group aged 0–2 months. The occurrence of dehydration decreased during the second year of life, and was very low among the older children. The distribution of rotavirus dehydrating episodes by age group was as follows: 6% (17/307) in infants aged 0–2 months; 21% (49/234) in infants aged 3–5 months; 22% (32/144) in children aged 6–8 months; 30% (29/96) children aged 9–11 months; and 16% (20/127) and 8% (1/12) in children older than 1 or 2 years of age, respectively.

Pattern of Immunity Acquisition

Rotavirus-specific immunity pattern was studied in 234 healthy infants <1 year of age (Fig. 4). Acquisition of specific IgA antibodies from exposure to rotavirus began very early in life (18% of infants 0–1 month of age were seropositive), gradually increasing by 6–11 months of life, when most infants (85%) have acquired rotavirus IgA antibody. With regard to neutralizing antibodies, 91% of infants aged 0–1 months had Wa neutralizing IgG antibody, most likely of predominantly maternal origin. Neutralizing antibodies declined gradually by 4–5 months of age, but by 6–11 months of age, the proportion of infants with such antibody increased markedly, as 100% of infants had antibody to Wa, reflecting recent exposure to natural infection.

DISCUSSION

Group A rotavirus disease is a worldwide public health problem in infants and young children. The most extensively evaluated rotavirus vaccine, a rhesus rotavirus-based quadrivalent formulation, was recently licensed by the U.S. Food and Drug Administration (FDA) for use in the United States. International license of rotavirus vaccine is anticipated in the near future. Thus, rotavirus epidemiology is emerging as an

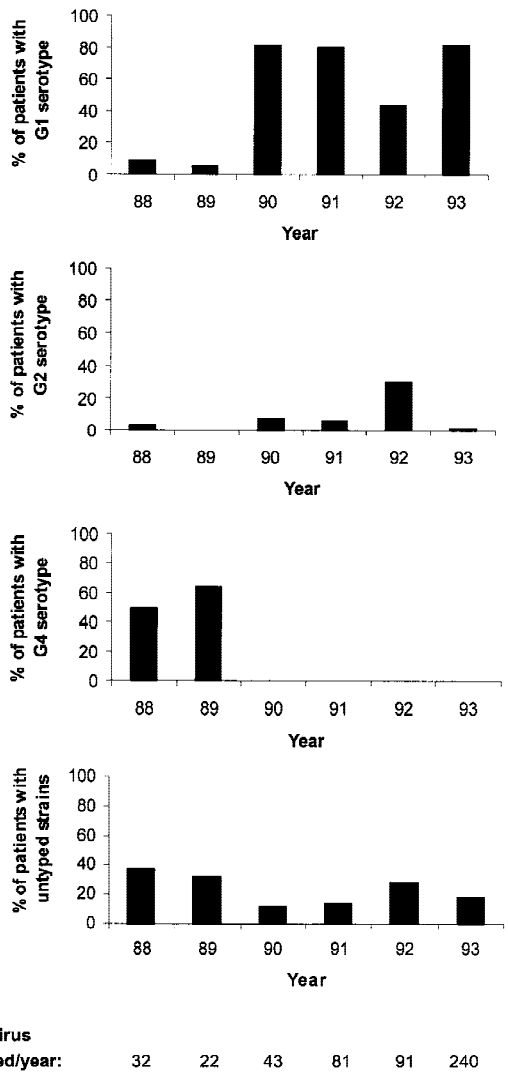

Fig. 3. Distribution of rotavirus G serotypes in Venezuela 1988–1993.

TABLE II. Clinical Characteristics of Infants and Children <3 Years of Age With Rotavirus and Nonrotavirus Diarrhea Who Attended to the Hospital Materno Infantil de Caricuao in Caracas, Venezuela: 1988–1993

Clinical characteristics of diarrheal episodes	No. of rotavirus episodes (%) 318 (35)	No. of nonrotavirus episodes (%) 602 (65)	P*
Dehydration	148 (47)	175 (29)	<0.001
Vomiting	252 (79)	283 (47)	<0.001
Fever	181 (57)	198 (33)	<0.001
Bloody stools	26 (8)	132 (22)	<0.001
Duration <7 days	284 (89)	500 (83)	0.01
Treatment			
Outpatient	77 (24)	286 (48)	<0.001
Inpatient	241 (76)	316 (52)	<0.001

*Fisher's exact test (two-tailed) for comparison of rotavirus versus nonrotavirus diarrheal episodes.

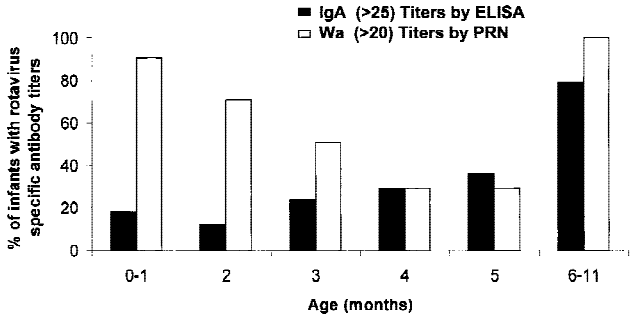

Fig. 4. Age distribution of rotavirus IgA and neutralizating (Wa) antibodies in serum from Venezuelan infants.

important element for designing effective vaccine programs and evaluating vaccine impact, particularly in developing countries.

Our results show that, in Venezuela, rotavirus is very common and of public health importance, accounting for 15–39% of all hospital-assisted diarrheal episodes. Rotavirus illness was more severe and significantly more frequently associated with hospitalization (76%), dehydration (47%), vomiting (79%), and fever (57%) than diarrhea caused by other agents ($P < 0.001$). Moreover, 85% of the episodes and 86% (127/148) of rotavirus dehydrating cases occurred in children younger than 1 year of age, indicating that early infection is very frequent in this setting. The prevalence of rotavirus in Caricuao was high regardless of socioeconomic conditions; however, severe rotavirus illness was more common in the lower socioeconomic groups. Our data confirm that rotavirus is a universal and ubiquitous disease. Furthermore, results corroborate previous studies that established rotavirus as the most common cause of severe illness, particularly during the first 2 years of life, although a less important cause of illness in older children [Bern and Glass, 1994; Ryan et al., 1996; Unicomb et al., 1997; Cook et al., 1990; Pérez-Schael, 1996].

Rotavirus illness occurred with lower frequency (16%) at 0–2 months of life, as compared with the group aged 3–11 months (47%). Studies showed similar results in other developing areas [Unicomb et al., 1997], as well as in developed countries [Ryan et al., 1996; Matson et al., 1990]. This protection may be due to transplacental antibody [Rojas et al., 1995] and/or breast-feeding [Rimer et al., 1992] or to age-dependent physiological factors [Bass et al., 1992]. The low incidence in the group aged 0–2 months is consistent with this pattern of immunity and with the age distribution of severe disease, supported by observations of natural infection [Rojas et al., 1995] or immunization studies [Pérez-Schael et al., 1997]. Our results suggest that maternal antibody is a major protective factor against rotavirus disease. On the other hand, our results showed that severe illness is rare in older children because the risk of severe disease diminishes with each rotavirus infections [Velázquez et al., 1996].

In this setting, rotavirus disease presented a slight seasonal pattern. Although present year-round, it was

significantly lower during the rainy and warmest months: April through June (13%), in contrast to other seasons (27–31%). Seasonal patterns are more evident in temperate climates [Torok et al., 1997, Ryan et al., 1996; Cook et al., 1990; Le Baron et al., 1990], whereas in tropical countries such as Venezuela [Viera de Torres et al., 1978], Bangladesh [Unicomb et al., 1997], or some regions from Brazil [Pereira et al., 1994], the disease occurs year-round, with minimal seasonality. This environmental phenomenon may be responsible for age-specific attack rates, since, in countries where a seasonal pattern does not occur, infants tend to become infected at an earlier age due to continual exposure to rotavirus than infants from regions in which seasonality is marked. In Venezuela, in spite of low seasonality and poor socioeconomic conditions, vaccine efficacy was high [Pérez-Schael et al., 1997], as compared with Brazil and Perú, where vaccine performance was poor [Linhares et al., 1996; Lanata et al., 1996]. Although, a lower dosis of the same vaccine was used in those countries, study design and/or age-specific rates of rotavirus diarrhea may be partly responsible for this difference [Pérez-Schael et al., 1997; Santosham et al., 1997; Glass et al., 1997; Linhares et al., 1996]. Crowding might also explain the low vaccine efficacy in Perú or Brazil, as it may induce a rapid spread of wild or vaccine viruses, thus masking the vaccine efficacy [Pérez-Schael et al., 1997].

An association between vaccine efficacy and age of vaccination has been demonstrated in Venezuela [Pericchi et al., 1989; Pérez-Schael et al., 1990]. The efficacy of one dose of rhesus (RRV) rotavirus vaccine was as follows: 34% in 1-month-old infants, and 93% and 0% of efficacy in infants aged 2–4 and 5–10 months, respectively [Pericchi et al., 1989]. Thus, rotavirus vaccine must be given early enough to prevent severe disease but, simultaneously, interference between vaccination and maternal antibodies must be averted. However, interference of maternal antibody with vaccine immunogenicity could be overcome by increasing the number of doses administered, as shown with the influence of breast-feeding and oral polio vaccine on rotavirus vaccine immunity [Rennels, 1996a]. On the other hand, natural immunity does interfere with vaccine efficacy [Pericchi et al., 1989]; thus, should be given before exposure to the wild virus. Consequently, high vaccine efficacy observed in Venezuela [Pérez-Schael et al., 1997] coincides with the age of vaccine administration (2–4 months) and this pattern of immunity. In conclusion, information about the natural history of rotavirus infection, especially in the developing countries, may be important for the implementation of rotavirus vaccination programs.

ACKNOWLEDGMENTS

We thank Dra. Ana María Rojas for performing the neutralization assays, Drs. Albert Kapikian and Joseph Bresee for review of the manuscript, and Dr. Laura Freimanis for reading the manuscript.

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